

**PCT**WORLD INTELLECTUAL PROPERTY ORGANIZATION  
International Bureau

## INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification <sup>6</sup> : <b>C12N 15/11, C07H 21/04, A61K 31/70 // 48/00</b>	<b>A1</b>	(11) International Publication Number: <b>WO 97/11170</b>
		(43) International Publication Date: 27 March 1997 (27.03.97)

(21) International Application Number: PCT/US96/15081

(22) International Filing Date: 20 September 1996 (20.09.96)

(30) Priority Data:

60/004,018

20 September 1995 (20.09.95) US

(71) Applicant (for all designated States except US): WORCES-  
TER FOUNDATION FOR BIOMEDICAL RESEARCH  
[US/US]; 222 Maple Street, Shrewsbury, MA 01545-8000  
(US).

(72) Inventor; and

(75) Inventor/Applicant (for US only): ZAMECNIK, Paul, A.  
[US/US]; 29 LeBeaux Drive, Shrewsbury, MA 01545 (US).(74) Agent: KINDREGAN, Helen; Wolf, Greenfield & Sacks, P.C.,  
600 Atlantic Avenue, Boston, MA 02210 (US).(81) Designated States: AU, CA, CN, JP, KP, NZ, US, European  
patent (AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT,  
LU, MC, NL, PT, SE).**Published***With international search report.**Before the expiration of the time limit for amending the  
claims and to be republished in the event of the receipt of  
amendments.*

(54) Title: ANTISENSE OLIGONUCLEOTIDE CHEMOTHERAPY FOR BENIGN HYPERPLASIA OR CANCER OF THE PROSTATE

## (57) Abstract

Methods of selectively inhibiting the growth of or killing prostatic cells, using antisense oligonucleotides to prostate specific genes, are disclosed. The oligonucleotides may have natural nucleic acid structures or may be modified oligonucleotides with enhanced stability or tissue specific targeting. The prostate specific genes to which the antisense may be directed include the AR and the  $\alpha$ FGF gene. Pharmaceutical compositions including such antisense oligonucleotides are also described for use in the methods. The methods and products are of particular utility in the treatment of benign prostatic hyperplasia or prostate cancer.

**FOR THE PURPOSES OF INFORMATION ONLY**

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AM	Armenia	GB	United Kingdom	MW	Malawi
AT	Austria	GE	Georgia	MX	Mexico
AU	Australia	GN	Guinea	NE	Niger
BB	Barbados	GR	Greece	NL	Netherlands
BE	Belgium	HU	Hungary	NO	Norway
BF	Burkina Faso	IE	Ireland	NZ	New Zealand
BG	Bulgaria	IT	Italy	PL	Poland
BJ	Benin	JP	Japan	PT	Portugal
BR	Brazil	KE	Kenya	RO	Romania
BY	Belarus	KG	Kyrgyzstan	RU	Russian Federation
CA	Canada	KP	Democratic People's Republic of Korea	SD	Sudan
CF	Central African Republic	KR	Republic of Korea	SE	Sweden
CG	Congo	KZ	Kazakhstan	SG	Singapore
CH	Switzerland	LI	Liechtenstein	SI	Slovenia
CI	Côte d'Ivoire	LK	Sri Lanka	SK	Slovakia
CM	Cameroon	LR	Liberia	SN	Senegal
CN	China	LT	Lithuania	SZ	Swaziland
CS	Czechoslovakia	LU	Luxembourg	TD	Chad
CZ	Czech Republic	LV	Latvia	TG	Togo
DE	Germany	MC	Monaco	TJ	Tajikistan
DK	Denmark	MD	Republic of Moldova	TT	Trinidad and Tobago
EE	Estonia	MG	Madagascar	UA	Ukraine
ES	Spain	ML	Mali	UG	Uganda
FI	Finland	MN	Mongolia	US	United States of America
FR	France	MR	Mauritania	UZ	Uzbekistan
GA	Gabon			VN	Viet Nam

**ANTISENSE OLIGONUCLEOTIDE CHEMOTHERAPY FOR  
BENIGN HYPERPLASIA OR CANCER OF THE PROSTATE**

5

10

15

20

25

30

5

10

15

20

### Summary of the Invention

The present invention provides methods for treating a patient diagnosed as having benign prostatic hyperplasia or a prostatic cancer. The methods include administering to the patient a therapeutically effective amount of a composition comprising an antisense oligonucleotide which  
25 selectively hybridizes to an AR or  $\alpha$ FGF gene or mRNA sequence of the patient, thereby inhibiting the expression of the AR or  $\alpha$ FGF gene or mRNA sequence. This inhibition of the AR or  $\alpha$ FGF genes or mRNAs by antisense oligonucleotides results in a significant inhibition of the growth or survival of prostatic cells. As a result, the methods provide a useful new means of treating benign prostatic hyperplasia and prostatic cancer. The methods are particularly useful in  
30 treating prostate cancer patients who have become refractory to anti-androgen hormonal therapy.

The AR antisense oligonucleotides may comprise at least 10 consecutive bases from SEQ

ID NO.: 1, at least 10 consecutive bases from a genomic sequence corresponding to SEQ ID NO.: 1, or oligonucleotides that hybridize to the complements of these sequences under physiological conditions. More preferably, the antisense oligonucleotides comprise at least 15 consecutive bases, and most preferably, 20-30 consecutive bases from the above-described sequences.

10

15

20

25

30

5

10

15

20

25

30

5

5

10

15

20

25

30

5

10

15

20

25

30



5

10

15

20

As will be understood by one of ordinary skill in the art, the antisense oligonucleotides of the present invention need not be perfectly complementary to the AR or  $\alpha$ FGF genes or mRNA transcripts in order to be effective. Rather, some degree of mismatches will be acceptable if the antisense oligonucleotide is of sufficient length.

25

30

Of particular importance in this respect is the use of self-stabilized or hairpin oligonucleotides. Such oligonucleotides, or modified oligonucleotides, have a sequence at the 5' and/or 3' end which is capable of folding over and forming a duplex with itself. The duplex region, which is preferably at least 4-6 bases joined by a loop of 3-6 bases, stabilizes the oligonucleotide against degradation. These self-stabilized oligonucleotides are easily designed by adding the inverted complement of a 5' or 3' AR or  $\alpha$ FGF sequence to the end of the oligonucleotide (see, e.g., Table 1, SEQ ID NO.: 6 and SEQ ID NO.: 7; Tang, J.-Y., et al. (1993) Nucleic Acids Res. 21:2729-2735).

10

15

20

25

30

5

10

15

20

25

30

5

10

15

20

25

30

5

10

15

20

25

30

5

10

15

20

25

30

In another series of embodiments, a recombinant gene is constructed which encodes an

AR or  $\alpha$ FGF antisense oligonucleotide and this gene is introduced within the targeted cells on a vector. Such an AR or  $\alpha$ FGF antisense gene may, for example, consist of the normal AR or  $\alpha$ FGF sequence, or a subset of the normal sequences, operably joined in reverse orientation to a promoter region. An operable antisense gene may be introduced on an integration vector or may  
5 be introduced on an expression vector. In order to be most effective, it is preferred that the antisense sequences be operably joined to a strong eukaryotic promoter which is inducible or constitutively expressed.

10

15

20

25

30

5

10

15

20

25

gene (SEQ ID NO.: 1). SEQ ID NO.: 6 is a self-stabilized or hairpin oligonucleotide. The first 21 bases are complementary to positions 916-936 of the AR gene. The remaining eight are identical to positions 920-927 of the gene, allowing formation of a 3' hairpin. SEQ ID NO.: 7 is another self-stabilized antisense oligonucleotide. The first 21 bases of this oligonucleotide are complementary to positions 927-947 of the AR gene. The remaining eight are identical to



positions 931-938 of the gene, allowing for formation of a 3' hairpin.

5

10

15

20

25

5

10

15

20

25

30

35

5

10

15

20

25

30

35

5

10

15

5

10

15

20

25

## SEQUENCE LISTING

## (1) GENERAL INFORMATION:

5

(i) APPLICANT: WORCESTER FOUNDATION FOR BIOMEDICAL RESEARCH, INC.

(ii) TITLE OF INVENTION: ANTISENSE OLIGONUCLEOTIDE CHEMOTHERAPY  
FOR BENIGN HYPERPLASIA OR CANCER OF THE PROSTATE

10

(iii) NUMBER OF SEQUENCES: 8

## (iv) CORRESPONDENCE ADDRESS:

15

(A) ADDRESSEE: WOLF, GREENFIELD &amp; SACKS, P.C.

(B) STREET: 600 ATLANTIC AVENUE

(C) CITY: BOSTON

(D) STATE: MA

(E) COUNTRY: USA

(F) ZIP: 02210

20

## (v) COMPUTER READABLE FORM:

(A) MEDIUM TYPE: Floppy disk

(B) COMPUTER: IBM PC compatible

(C) OPERATING SYSTEM: PC-DOS/MS-DOS

25

(D) SOFTWARE: PatentIn Release #1.0, Version #1.25

## (vi) CURRENT APPLICATION DATA:

(A) APPLICATION NUMBER:

(B) FILING DATE:

30

(C) CLASSIFICATION:

## (viii) ATTORNEY/AGENT INFORMATION:

- (A) NAME: TWOMEY, MICHAEL J.
- (B) REGISTRATION NUMBER: 38,349
- (C) REFERENCE/DOCKET NUMBER: W0461/7035

5

## (ix) TELECOMMUNICATION INFORMATION:

- (A) TELEPHONE: 617-720-3500
- (B) TELEFAX: 617-720-2441

10

## (2) INFORMATION FOR SEQ ID NO:1:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 3569 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: double
- (D) TOPOLOGY: linear

15

## (ii) MOLECULE TYPE: cDNA

20

## (iii) HYPOTHETICAL: NO

## (iv) ANTI-SENSE: NO

25

## (vi) ORIGINAL SOURCE:

- (A) ORGANISM: HOMO SAPIENS

## (ix) FEATURE:

- (A) NAME/KEY: CDS
- (B) LOCATION: 363..3122

30

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

35 TAATAACTCA GTTCTTATTT GCACCTACTT CAGTGGACAC TGAATTGGA AGGTGGAGGA 60

TTTTGTTTTT TTCTTTTAAG ATCTGGGCAT CTTTGAATC TACCCTTCAA GTATTAAGAG 120  
ACAGACTGTG AGCCTAGCAG GGCAGATCTT GTCCACCGTG TGTCTTCTTC TGCACGAGAC 180  
5 TTTGAGGCTG TCAGAGCGCT TTTTGCCTGG TTGCTCCCGC AAGTTTCCTT CTCTGGAGCT 240  
TCCCGCAGGT GGGCAGCTAG CTGCAGCGAC TACCGCATCA TCACAGCCTG TTGAACTCTT 300  
CTGAGCAAGA GAAGGGGAGG CGGGGTAAGG GAAGTAGGTG GAAGATTCAG CCAAGCTCAA 360  
10 GG ATG GAA GTG CAG TTA GGG CTG GGA AGG GTC TAC CCT CGG CCG CCG 407  
Met Glu Val Gln Leu Gly Leu Gly Arg Val Tyr Pro Arg Pro Pro  
1 5 10 15  
15 TCC AAG ACC TAC CGA GGA GCT TTC CAG AAT CTG TTC CAG AGC GTG CGC 455  
Ser Lys Thr Tyr Arg Gly Ala Phe Gln Asn Leu Phe Gln Ser Val Arg  
20 25 30  
GAA GTG ATC CAG AAC CCG GGC CCC AGG CAC CCA GAG GCC GCG AGC GCA 503  
20 Glu Val Ile Gln Asn Pro Gly Pro Arg His Pro Glu Ala Ala Ser Ala  
35 40 45  
GCA CCT CCC GGC GCC AGT TTG CTG CTG CTG CAG CAG CAG CAG CAG 551  
Ala Pro Pro Gly Ala Ser Leu Leu Leu Leu Gln Gln Gln Gln Gln Gln  
25 50 55 60



CAG CAG CAG CAG CAG CAG CAG CAG CAG CAG CAG CAG CAG CAG CAA GAG 599  
 Gln Gln Gln Gln Gln Gln Gln Gln Gln Gln Gln Gln Gln Gln Gln Glu  
 65 70 75

5 ACT AGC CCC AGG CAG CAG CAG CAG CAG CAG GGT GAG GAT GGT TCT CCC 647  
 Thr Ser Pro Arg Gln Gln Gln Gln Gln Gln Gly Glu Asp Gly Ser Pro  
 80 85 90 95

CAA GCC CAT CGT AGA GGC CCC ACA GGC TAC CTG GTC CTG GAT GAG GAA 695  
 10 Gln Ala His Arg Arg Gly Pro Thr Gly Tyr Leu Val Leu Asp Glu Glu  
 100 105 110

CAG CAA CCT TCA CAG CCG CAG TCG GCC CTG GAG TGC CAC CCC GAG AGA 743  
 Gln Gln Pro Ser Gln Pro Gln Ser Ala Leu Glu Cys His Pro Glu Arg  
 15 115 120 125

GGT TGC GTC CCA GAG CCT GGA GCC GCC GTG GCC GCC AGC AAG GGG CTG 791  
 Gly Cys Val Pro Glu Pro Gly Ala Ala Val Ala Ala Ser Lys Gly Leu  
 130 135 140

20 CCG CAG CAG CTG CCA GCA CCT CCG GAC GAG GAT GAC TCA GCT GCC CCA 839  
 Pro Gln Gln Leu Pro Ala Pro Pro Asp Glu Asp Asp Ser Ala Ala Pro  
 145 150 155

25 TCC ACG TTG TCC CTG CTG GGC CCC ACT TTC CCC GGC TTA AGC AGC TGC 887  
 Ser Thr Leu Ser Leu Leu Gly Pro Thr Phe Pro Gly Leu Ser Ser Cys  
 160 165 170 175

TCC GCT GAC CTT AAA GAC ATC CTG AGC GAG GCC AGC ACC ATG CAA CTC 935  
 30 Ser Ala Asp Leu Lys Asp Ile Leu Ser Glu Ala Ser Thr Met Gln Leu  
 180 185 190

CTT CAG CAA CAG CAG CAG GAA GCA GTA TCC GAA GGC AGC AGC AGC GGG 983  
 Leu Gln Gln Gln Gln Gln Glu Ala Val Ser Glu Gly Ser Ser Ser Gly  
 35 195 200 205

AGA GCG AGG GAG GCC TCG GGG GCT CCC ACT TCC TCC AAG GAC AAT TAC 1031  
 Arg Ala Arg Glu Ala Ser Gly Ala Pro Thr Ser Ser Lys Asp Asn Tyr  
 210 215 220

5 TTA GGG GGC ACT TCG ACC ATT TCT GAC AAC GCC AAG GAG TTG TGT AAG 1079  
 Leu Gly Gly Thr Ser Thr Ile Ser Asp Asn Ala Lys Glu Leu Cys Lys  
 225 230 235

GCA GTG TCG GTG TCC ATG GGC CTG GGT GTG GAG GCG TTG GAG CAT CTG 1127  
 10 Ala Val Ser Val Ser Met Gly Leu Gly Val Glu Ala Leu Glu His Leu  
 240 245 250 255

AGT CCA GGG GAA CAG CTT CGG GGG GAT TGC ATG TAC GCC CCA CTT TTG 1175  
 Ser Pro Gly Glu Gln Leu Arg Gly Asp Cys Met Tyr Ala Pro Leu Leu  
 15 260 265 270

GGA GTT CCA CCC GCT GTG CGT CCC ACT CCT TGT GCC CCA TTG GCC GAA 1223  
 Gly Val Pro Pro Ala Val Arg Pro Thr Pro Cys Ala Pro Leu Ala Glu  
 275 280 285

20 TGC AAA GGT TCT CTG CTA GAC GAC AGC GCA GGC AAG AGC ACT GAA GAT 1271  
 Cys Lys Gly Ser Leu Leu Asp Asp Ser Ala Gly Lys Ser Thr Glu Asp  
 290 295 300

25 ACT GCT GAG TAT TCC CCT TTC AAG GGA GGT TAC ACC AAA GGG CTA GAA 1319  
 Thr Ala Glu Tyr Ser Pro Phe Lys Gly Gly Tyr Thr Lys Gly Leu Glu  
 305 310 315

GGC GAG AGC CTA GGC TGC TCT GGC AGC GCT GCA GCA GGG AGC TCC GGG 1367  
 30 Gly Glu Ser Leu Gly Cys Ser Gly Ser Ala Ala Ala Gly Ser Ser Gly  
 320 325 330 335

ACA CTT GAA CTG CCG TCT ACC CTG TCT CTC TAC AAG TCC GGA GCA CTG 1415  
 Thr Leu Glu Leu Pro Ser Thr Leu Ser Leu Tyr Lys Ser Gly Ala Leu  
 35 340 345 350

GAC GAG GCA GCT GCG TAC CAG AGT CGC GAC TAC TAC AAC TTT CCA CTG 1463  
 Asp Glu Ala Ala Ala Tyr Gln Ser Arg Asp Tyr Tyr Asn Phe Pro Leu  
 355 360 365

5 GCT CTG GCC GGA CCG CCG CCC CCT CCG CCG CCT CCC CAT CCC CAC GCT 1511  
 Ala Leu Ala Gly Pro Pro Pro Pro Pro Pro Pro Pro His Pro His Ala  
 370 375 380

CGC ATC AAG CTG GAG AAC CCG CTG GAC TAC GGC AGC GCC TGG GCG GCT 1559  
 10 Arg Ile Lys Leu Glu Asn Pro Leu Asp Tyr Gly Ser Ala Trp Ala Ala  
 385 390 395

GCG GCG GCG CAG TGC CGC TAT GGG GAC CTG GCG AGC CTG CAT GGC GCG 1607  
 Ala Ala Ala Gln Cys Arg Tyr Gly Asp Leu Ala Ser Leu His Gly Ala  
 15 400 405 410 415

GGT GCA GCG GGA CCC GGT TCT GGG TCA CCC TCA GCC GCC GCT TCC TCA 1655  
 Gly Ala Ala Gly Pro Gly Ser Gly Ser Pro Ser Ala Ala Ala Ser Ser  
 420 425 430

20 TCC TGG CAC ACT CTC TTC ACA GCC GAA GAA GGC CAG TTG TAT GGA CCG 1703  
 Ser Trp His Thr Leu Phe Thr Ala Glu Glu Gly Gln Leu Tyr Gly Pro  
 435 440 445

TGT GGT GGT GGT GGG GGT GGT GGC GGC GGC GGC GGC GGC GGC GGC 1751  
 Cys Gly Gly Gly Gly Gly Gly Gly Gly Gly Gly Gly Gly Gly Gly Gly  
 450 455 460

GGC GGC GGC GGC GGC GGC GGC GGC GGC GAG GCG GGA GCT GTA GCC CCC 1799  
 30 Gly Gly Gly Gly Gly Gly Gly Gly Gly Gly Glu Ala Gly Ala Val Ala Pro  
 465 470 475

TAC GGC TAC ACT CGG CCC CCT CAG GGG CTG GCG GGC CAG GAA AGC GAC 1847  
 Tyr Gly Tyr Thr Arg Pro Pro Gln Gly Leu Ala Gly Gln Glu Ser Asp  
 35 480 485 490 495

TTC ACC GCA CCT GAT GTG TGG TAC CCT GGC GGC ATG GTG AGC AGA GTG 1895  
 Phe Thr Ala Pro Asp Val Trp Tyr Pro Gly Gly Met Val Ser Arg Val  
 500 505 510

5 CCC TAT CCC AGT CCC ACT TGT GTC AAA AGC GAA ATG GGC CCC TGG ATG 1943  
 Pro Tyr Pro Ser Pro Thr Cys Val Lys Ser Glu Met Gly Pro Trp Met  
 515 520 525

GAT AGC TAC TCC GGA CCT TAC GGG GAC ATG CGT TTG GAG ACT GCC AGG 1991  
 10 Asp Ser Tyr Ser Gly Pro Tyr Gly Asp Met Arg Leu Glu Thr Ala Arg  
 530 535 540

GAC CAT GTT TTG CCC ATT GAC TAT TAC TTT CCA CCC CAG AAG ACC TGC 2039  
 Asp His Val Leu Pro Ile Asp Tyr Tyr Phe Pro Pro Gln Lys Thr Cys  
 15 545 550 555

CTG ATC TGT GGA GAT GAA GCT TCT GGG TGT CAC TAT GGA GCT CTC ACA 2087  
 Leu Ile Cys Gly Asp Glu Ala Ser Gly Cys His Tyr Gly Ala Leu Thr  
 560 565 570 575

20 TGT GGA AGC TGC AAG GTC TTC TTC AAA AGA GCC GCT GAA GGG AAA CAG 2135  
 Cys Gly Ser Cys Lys Val Phe Phe Lys Arg Ala Ala Glu Gly Lys Gln  
 580 585 590

25 AAG TAC CTG TGC GCC AGC AGA AAT GAT TGC ACT ATT GAT AAA TTC CGA 2183  
 Lys Tyr Leu Cys Ala Ser Arg Asn Asp Cys Thr Ile Asp Lys Phe Arg  
 595 600 605

AGG AAA AAT TGT CCA TCT TGT CGT CTT CGG AAA TGT TAT GAA GCA GGG 2231  
 30 Arg Lys Asn Cys Pro Ser Cys Arg Leu Arg Lys Cys Tyr Glu Ala Gly  
 610 615 620

ATG ACT CTG GGA GCC CGG AAG CTG AAG AAA CTT GGT AAT CTG AAA CTA 2279  
 Met Thr Leu Gly Ala Arg Lys Leu Lys Lys Leu Gly Asn Leu Lys Leu  
 35 625 630 635

CAG GAG GAA GGA GAG GCT TCC AGC ACC ACC AGC CCC ACT GAG GAG ACA 2327  
 Gln Glu Glu Gly Glu Ala Ser Ser Thr Thr Ser Pro Thr Glu Glu Thr  
 640 645 650 655

5 ACC CAG AAG CTG ACA GTG TCA CAC ATT GAA GGC TAT GAA TGT CAG CCC 2375  
 Thr Gln Lys Leu Thr Val Ser His Ile Glu Gly Tyr Glu Cys Gln Pro  
 660 665 670

ATC TTT CTG AAT GTC CTG GAA GCC ATT GAG CCA GGT GTA GTG TGT GCT 2423  
 10 Ile Phe Leu Asn Val Leu Glu Ala Ile Glu Pro Gly Val Val Cys Ala  
 675 680 685

GGA CAC GAC AAC AAC CAG CCC GAC TCC TTT GCA GCC TTG CTC TCT AGC 2471  
 Gly His Asp Asn Asn Gln Pro Asp Ser Phe Ala Ala Leu Leu Ser Ser  
 15 690 695 700

CTC AAT GAA CTG GGA GAG AGA CAG CTT GTA CAC GTG GTC AAG TGG GCC 2519  
 Leu Asn Glu Leu Gly Glu Arg Gln Leu Val His Val Val Lys Trp Ala  
 705 710 715

20 AAG GCC TTG CCT GGC TTC CGC AAC TTA CAC GTG GAC GAC CAG ATG GCT 2567  
 Lys Ala Leu Pro Gly Phe Arg Asn Leu His Val Asp Asp Gln Met Ala  
 720 725 730 735

25 GTC ATT CAG TAC TCC TGG ATG GGG CTC ATG GTG TTT GCC ATG GGC TGG 2615  
 Val Ile Gln Tyr Ser Trp Met Gly Leu Met Val Phe Ala Met Gly Trp  
 740 745 750

CGA TCC TTC ACC AAT GTC AAC TCC AGG ATG CTC TAC TTC GCC CCT GAT 2663  
 30 Arg Ser Phe Thr Asn Val Asn Ser Arg Met Leu Tyr Phe Ala Pro Asp  
 755 760 765

CTG GTT TTC AAT GAG TAC CGC ATG CAC AAG TCC CGG ATG TAC AGC CAG 2711  
 Leu Val Phe Asn Glu Tyr Arg Met His Lys Ser Arg Met Tyr Ser Gln  
 35 770 775 780

TGT GTC CGA ATG AGG CAC CTC TCT CAA GAG TTT GGA TGG CTC CAA ATC 2759  
 Cys Val Arg Met Arg His Leu Ser Gln Glu Phe Gly Trp Leu Gln Ile  
 785 790 795

5 ACC CCC CAG GAA TTC CTG TGC ATG AAA GCA CTG CTA CTC TTC AGC ATT 2807  
 Thr Pro Gln Glu Phe Leu Cys Met Lys Ala Leu Leu Leu Phe Ser Ile  
 800 805 810 815

ATT CCA GTG GAT GGG CTG AAA AAT CAA AAA TTC TTT GAT GAA CTT CGA 2855  
 10 Ile Pro Val Asp Gly Leu Lys Asn Gln Lys Phe Phe Asp Glu Leu Arg  
 820 825 830

ATG AAC TAC ATC AAG GAA CTC GAT CGT ATC ATT GCA TGC AAA AGA AAA 2903  
 Met Asn Tyr Ile Lys Glu Leu Asp Arg Ile Ile Ala Cys Lys Arg Lys  
 15 835 840 845

AAT CCC ACA TCC TGC TCA AGA CGC TTC TAC CAG CTC ACC AAG CTC CTG 2951  
 Asn Pro Thr Ser Cys Ser Arg Arg Phe Tyr Gln Leu Thr Lys Leu Leu  
 850 855 860

20 GAC TCC GTG CAG CCT ATT GCG AGA GAG CTG CAT CAG TTC ACT TTT GAC 2999  
 Asp Ser Val Gln Pro Ile Ala Arg Glu Leu His Gln Phe Thr Phe Asp  
 865 870 875

25 CTG CTA ATC AAG TCA CAC ATG GTG AGC GTG GAC TTT CCG GAA ATG ATG 3047  
 Leu Leu Ile Lys Ser His Met Val Ser Val Asp Phe Pro Glu Met Met  
 880 885 890 895

GCA GAG ATC ATC TCT GTG CAA GTG CCC AAG ATC CTT TCT GGG AAA GTC 3095  
 30 Ala Glu Ile Ile Ser Val Gln Val Pro Lys Ile Leu Ser Gly Lys Val  
 900 905 910

AAG CCC ATC TAT TTC CAC ACC CAG TGAAGCATTG GAAACCCTAT TTCCCCACCC 3149  
 Lys Pro Ile Tyr Phe His Thr Gln  
 35 915 920

CAGCTCATGC CCCCTTTCAG ATGTCTTCTG CCTGTTATAA CTCTGCACTA CTCCTCTGCA 3209

GTGCCTTGGG GAATTCCTC TATTGATGTA CAGTCTGTCA TGAACATGTT CCTGAATTCT 3269

5 ATTTGCTGGG CTTTTTTTTT CTCTTTCTCT CCTTTCTTTT TCTTCTTCCC TCCCTATCTA 3329

ACCCTCCCAT GGCACCTTCA GACTTTGCTT CCCATTGTGG CTCCTATCTG TGTTTTGAAT 3389

GGTGTGTAT GCCTTTAAAT CTGTGATGAT CCTCATATGG CCCAGTGTCA AGTTGTGCTT 3449

10

GTTTACAGCA CTACTCTGTG CCAGCCACAC AAACGTTTAC TTATCTTATG CCACGGGAAG 3509

TTTAGAGAGC TAAGATTATC TGGGGAAATC AAAACAAAAA ACAAGCAAAC AAAAAAAAAA 3569

15

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 1082 base pairs

20

(B) TYPE: nucleic acid

(C) STRANDEDNESS: double

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

25

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

30

(vi) ORIGINAL SOURCE:

(A) ORGANISM: HOMO SAPIENS

(ix) FEATURE:

(A) NAME/KEY: exon

35

(B) LOCATION: 602..770

(D) OTHER INFORMATION: /note= "SEGMENT 1 OF 3."



(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

AAGCTTCCCT TAACATACTA ACCCTTTACT TTCCCTGTTG TGTCCCTGAA AGGCCTCCTG 60

5 TGCCTTTGGC TGCAGGTCCC GAACGTCCAG GCCATCTGTG CTATCTGCTT CGCGGTACCT 120

CACCAACGCA ACGTGAGGGT GGAGGGCAGA ACCTTGGTCC TGGCCTCTCA GCTTTTGTGG 180

GTTTCAGCCA GACCCTAGGT GTTATTTTAG TGCAACTTTG GTGTTTAATT TGAGGATGTG 240

10 TGTGGACCAG AAGGAGGGAC CAAAACATGA TTCTTTTCCC CATGGTCAGA TGATTAAATT 300

TGAAGTTCTA AAAAATGCAG TTTGGTCCAA AGCTGTGTCC AATTGGGAAG AGAGAAAAAT 360

15 GCCCTGGAAA CCCCTCCCAG GCCTGGGACC ATCCTTCCTT AACCACCAGC CACCTCACAG 420

GCCCGCGGAC TGCGGGCATC ACCTGGGCAG GCTGTGCTTA CTCACTACCC GGGAACCCTG 480

TGCCCTGGAG CTGTCCTTCC TCTCTTCAAA GTGCATTTTG TGCCTTTGCT GGAAGAACCG 540

20 ACTACAGGTT TGTTCATTTT CTTACAGTCT TGAAAGCGCC ACAAGCAGCA GCTGCTGAGC 600

CATGGCTGAA GGGGAAATCA CCACCTTCAC AGCCCTGACC GAGAAGTTTA ATCTGCCTCC 660

25 AGGGAATTAC AAGAAGCCCA AACTCCTCTA CTGTAGCAAC GGGGGCCACT TCCTGAGGAT 720

CCTTCCGGAT GGCACAGTGG ATGGGACAAG GGACAGGAGC GACCAGCACA GTAAGCCCAT 780

CTCTATGGCA CCCCCCTTCC CTTTCTGACA TCTTCTGTAG TCAAGGTGGG AGGAAGGTGC 840

30 ACATTTAAGT ACAGGTACTT GCTTCTCCAA GGTTCATTC AGGCATGACA CATTCAGAGG 900

TGGAGTCACA TAAATGCGTA AAATGTCTGG GAAATGAAAA TAGGGACTTG TGGGGGCCAC 960

35 CACTTACCCA AACGTGTCCT ATTTCAAGTT TTTTAAAGCA CTCTCTGCTG ACCCAACAGA 1020

ACGGGCTGCC GGTGCTCAAT TGCTGTATGT TTTCCCAGGT TTCTGTAAGT AGTGAAAGAT 1080

CT

1082

5 (2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 427 base pairs

(B) TYPE: nucleic acid

10 (C) STRANDEDNESS: double

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

15 (iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:

20 (A) ORGANISM: HOMO SAPIENS

(ix) FEATURE:

(A) NAME/KEY: exon

(B) LOCATION: 186..289

25 (D) OTHER INFORMATION: /note= "SEGMENT 2 OF 3. UNKNOWN  
NUMBER OF BP AFTER SEGMENT 1."

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

30

CAGCTTTCTT TGGAAGGCAA AGAAAAAGGG ACTGTATTTC TATGTTTGA TTAATCTGAG 60

GCTCATCCTG AGGGCTCCGT GAAATGAATG AGCAGAATT TCCATGGCCA ACTGTCCTGG 120

35 CTGCCGGGTC CTATCGGCAA AAGCGTAGTG TTTATTTACT TTTGCTCGTG TTATTTTAT 180

TCCAGTTCAG CTGCAGCTCA GTGCGGAAAG CGTGGGGGAG GTGTATATAA AGAGTACCGA 240

GACTGGCCAG TACTTGGCCA TGGACACCGA CGGGCTTTTA TACGGCTCAG TAAGTATGAA 300

5 GCTGACATGC TTCCAGACGT TGGCCAAGGT TTGAGGTTTC CAGAAATCTT GTTACATGGA 360

GTGAGGCAAA CTATAAAGCA ACAATTAGTC TCTGTTTGTT ATTTTTCCTCA GAAGGATTCC 420

CACCCTC 427

10

(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:

- 15 (A) LENGTH: 664 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: double  
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

20

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

25

(vi) ORIGINAL SOURCE:

(A) ORGANISM: HOMO SAPIENS

(ix) FEATURE:

- 30 (A) NAME/KEY: exon  
(B) LOCATION: 304..498  
(D) OTHER INFORMATION: /note= "SEGMENT 3 OF 3. UNKNOWN  
NUMBER OF BP AFTER SEGMENT 2."

35

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

TGAGGACTCT TAGAAGTGCT CTTATCAGTA GCATCTTAAT TACTTTACAA TGGATTTTAA 60  
ATGGAAAGGA AGTTTACAAT AATAGCAAAT GCATATTGAC AGCTCTTTAG TGCCCGGTGC 120  
5 TGTCTAAGT CTTATGACT ACCCTGTGAA ATAAGTTCCA CCATGACCCC AATTTTCCTG 180  
AAAAGGAGAC TGAGGCATCG AGAGCTTTAG TATTTTGCCC AATGTCACAC AGCTAGTAAA 240  
TGGGGACCCC CATGTGAAAC TACTCACTGA TTGTCCTACT CTCTTGTTGGT TTTATCTTTT 300  
10 TAGCAGACAC CAAATGAGGA ATGTTTGTTT CTGGAAAGGC TGGAGGAGAA CCATTACAAC 360  
ACCTATATAT CCAAGAAGCA TGCAGAGAAG AATTGGTTTG TTGGCCTCAA GAAGAATGGG 420  
15 AGCTGCAAAC GCGGTCCTCG GACTCACTAT GGCCAGAAAG CAATCTTGTT TCTCCCCCTG 480  
CCAGTCTCTT CTGATTAAAG AGATCTGTTC TGGGTGTTGA CCACTCCAGA GAAGTTTCGA 540  
GGGGTCCTCA CCTGGTTGAC CCAAAAATGT TCCCTTGACC ATTGGCTGCG CTAACCCCCA 600  
20 GCCCACAGAG CCTGAATTTG TAAGCAACTT GCTTCTAAAT GCCCAGTTCA CTTCTTTGCA 660  
GAGC 664

25 (2) INFORMATION FOR SEQ ID NO:5:

(i) SEQUENCE CHARACTERISTICS:

- 30 (A) LENGTH: 27 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

35 (iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: YES

(vi) ORIGINAL SOURCE:

(A) ORGANISM: SYNTHETIC OLIGONUCLEOTIDE

5

(ix) FEATURE:

(A) NAME/KEY: misc\_feature

(B) LOCATION: 1..27

(D) OTHER INFORMATION: /note= "ANTISENSE TO POSITIONS  
927-953 OF SEQ ID NO.: 1."

10

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

15 CTGCTGCTGT TGCTGAAGGA GTTGCAT

27

(2) INFORMATION FOR SEQ ID NO:6:

(i) SEQUENCE CHARACTERISTICS:

20

(A) LENGTH: 29 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

25

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: YES

30

(vi) ORIGINAL SOURCE:

(A) ORGANISM: SYNTHETIC OLIGONUCLEOTIDE

(ix) FEATURE:

35

(A) NAME/KEY: misc\_feature

(B) LOCATION: 1..21

(D) OTHER INFORMATION: /note= "ANTISENSE TO POSITIONS  
916-936 OF SEQ ID NO.: 1."

5

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

GGAGTTGCAT GGTGCTGGCC TCAGCACCA

29

10 (2) INFORMATION FOR SEQ ID NO:7:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 29 base pairs

(B) TYPE: nucleic acid

15 (C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

20 (iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: YES

(vi) ORIGINAL SOURCE:

25 (A) ORGANISM: SYNTHETIC OLIGONUCLEOTIDE

(ix) FEATURE:

(A) NAME/KEY: misc\_feature

(B) LOCATION: 1..21

30 (D) OTHER INFORMATION: /note= "ANTISENSE TO POSITIONS  
927-947 OF SEQ ID NO.: 1."

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

35

CTGTTGCTGA AGGAGTTGCA TAACTCCTT

29

## (2) INFORMATION FOR SEQ ID NO:8:

## 5 (i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 25 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

10

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

15 (iv) ANTI-SENSE: YES

(vi) ORIGINAL SOURCE:

(A) ORGANISM: SYNTHETIC OLIGONUCLEOTIDE

20 (ix) FEATURE:

(A) NAME/KEY: misc\_feature

(B) LOCATION: 1..25

(D) OTHER INFORMATION: /note= "ANTISENSE TO POSITIONS  
611-635 OF SEQ ID NO.: 2."

25

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

GGGCTGTGAA GGTGGTGATT TCCCC

25

30

5

10

15

20

25



5

10

15

20

25

30

5

10

15

20

25

30

5

10

15

20

25

30

5

10

15

20

25

30

5

10

## INTERNATIONAL SEARCH REPORT

International Application No.  
PCT/US 96/15081

## A. CLASSIFICATION OF SUBJECT MATTER

IPC 6 C12N15/11 C07H21/04 A61K31/70 //A61K48/00

According to International Patent Classification (IPC) or to both national classification and IPC

## B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 6 C12N C07H A61K C07K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

## C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 94 05268 A (BAYLOR COLLEGE MEDICINE) 17 March 1994  see page 8, line 14 - page 10, line 20 see example 1 see claims 1,2,17-21,32-35 ---	1,7,13, 15,21, 28,29
X	WO 89 09791 A (UNIV NORTH CAROLINA) 19 October 1989 see page 2, line 12 - line 32 see page 24 ---	1,28,29
X	WO 95 11301 A (UNIV MICHIGAN) 27 April 1995 see claims ---	28,29
	-/-	

☒ Further documents are listed in the continuation of box C.☒ Patent family members are listed in annex.

## \* Special categories of cited documents:

- \*A\* document defining the general state of the art which is not considered to be of particular relevance
- \*E\* earlier document but published on or after the international filing date
- \*L\* document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
- \*O\* document referring to an oral disclosure, use, exhibition or other means
- \*P\* document published prior to the international filing date but later than the priority date claimed

- \*T\* later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
- \*X\* document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
- \*Y\* document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
- \*Z\* document member of the same patent family

Date of the actual completion of the international search

14 February 1997

Date of mailing of the international search report

26.02.97

Name and mailing address of the ISA

European Patent Office, P.B. 5818 Patentlaan 2  
NL - 2280 HV Rijswijk  
Tel. (+31-70) 340-2040, Tx. 31 651 epo nl.  
Fax (+31-70) 340-3016

Authorized officer

Andres, S

## INTERNATIONAL SEARCH REPORT

International Application No.

PCT/US 96/15081

## C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	CANCER RESEARCH, (1994 MAY 1) 54 (9) 2372-7., XP002025258 ACHBAROU, A. ET AL.: "Urokinase overproduction results in increased skeletal metastasis by prostate cancer cells in vivo." see the whole document ---	28,29
A	CANCER SURVEYS, vol. 11, 1991, pages 239-254, XP000616360 SHERIDAN, V. & TEW, K.: "Mechanism based chemotherapy for prostate cancer" cited in the application see the whole document ---	12,26
O,A	ANTISENSE RES.DEV. 5 ( FALL 1995); PAGE 239; ABSTRACT III12, XP002025259 HEAD, M. ET AL.: "Penetration and stability of antisense oligonucleotides injected into the early embryonic chick eye" see abstract & INT.CONF.: 'THERAPEUTIC OLIGONUCLEOTIDES FROM CELL TO MAN'; 4 TO 7 APRIL 1995; SEILLAC; FRANCE, ---	1,4-9
P,X	US 5 556 956 A (ROY ARUN K ET AL) 17 September 1996 --- see the whole document	1,7-10, 13,15, 21-24, 27-29
P,X	CELL GROWTH AND DIFFERENTIATION, (1996 MAY) 7 (5) 573-86., XP000616505 SHAIN, S. ET AL.: "Endogenous fibroblast growth factor - 1 or fibroblast growth factor -2 modulate prostate cancer cell proliferation." see the whole document ---	1,4-9, 28,29
P,X	JOURNAL OF BIOLOGICAL CHEMISTRY, (1996 MAY 31) 271 (22) 13228-33., XP002025260 BOFFA, L. ET AL.: "Invasion of the CAG triplet repeats by a complementary peptide nucleic acid inhibits transcription of the androgen receptor and TATA-binding protein genes and correlates with refolding of an active nucleosome containing a unique AR gene sequence." see the whole document ---	1-3,7,8, 10,28,29
1 3 P,X P,A	WO 96 03875 A (UNIV EMORY) 15 February 1996 see page 11, line 12 - page 13, line 21 ---	28,29 12,26
	--- -/--	

# INTERNATIONAL SEARCH REPORT

International Application No  
PCT/US 96/15081

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT		
Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
O,P, X	<p>PROCEEDINGS OF THE AMERICAN ASSOCIATION FOR CANCER RESEARCH ANNUAL MEETING 37 (0), March 1996, page 344 XP002025261 STEINER, M. ET AL.: "Gene therapy of advanced prostate cancer by in vivo transduction with prostate-targeted antisense c- myc RNA retroviruses." see abstract #2349 &amp; 87TH ANNUAL MEETING OF THE AMERICAN ASSOCIATION FOR CANCER RESEARCH, WASHINGTON, D.C., USA, APRIL 20-24, 1996., -----</p>	28,29



# INTERNATIONAL SEARCH REPORT

Intern: al application No.

PCT/US 96/ 15081

## Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☒ Claims Nos.:  
because they relate to subject matter not required to be searched by this Authority, namely:  
Please see Further Information sheet enclosed.
2. ☐ Claims Nos.:  
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:
3. ☐ Claims Nos.:  
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

## Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest.
- ☐ No protest accompanied the payment of additional search fees.

# INTERNATIONAL SEARCH REPORT

International Application No. PCT/US 96/ 15081

FURTHER INFORMATION CONTINUED FROM PCT/ISA/210

Remark : Although claims 1-14, 28-29 (as far as in vivo methods are concerned) are directed to a method of treatment of (diagnostic method practised on) the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.

# INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No

PCT/US 96/15081

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO-A-9405268	17-03-94	AU-A- 4846793	29-03-94
WO-A-8909791	19-10-89	EP-A- 0365657	02-05-90
WO-A-9511301	27-04-95	AU-A- 7983294	08-05-95
US-A-5556956	17-09-96	NONE	
WO-A-9603875	15-02-96	AU-A- 3071995	04-03-96